We explore the ultrastructure of the well-studied *Drosophilamelanogaster-gypsy* chromatin insulator by immunolabeling a key insulator protein CP190 using a fluoronanogold conjugated antibody probe. In our correlative method, fluorescent imaging is initially performed to identify nuclei that contain insulator bodies, which are rare within thin sections. A comparison of low-magnification EM image of a whole cell with the corresponding fluorescent image reveals the approximate location of the structure of interest. The fluorescence signal observed by light microscope guarantees the presence of the conjugated nanogold, which can be visualized using STEM, and used to locate precisely the labeled CP190 proteins. EFTEM is then performed to image the distribution of nitrogen and phosphorus and thus map the distributions of protein and nucleic acid. It is evident from these two elemental maps that the insulator body contains an abundance of protein but a small quantity of nucleic acid. Even though dense chromatin surrounds the insulator body, it is difficult to determine whether the low levels of phosphorus within the insulator body structures correspond to DNA or RNA, which requires further investigation.

3333-Pos Board B438

Dominant Vinculin Binding Angle in Podosomes Revealed by High Resolution Optical Microscopy

Susan Cox, Marie Walde, James Monypenny, Rainer Heintzmann, Gareth Jones.

Podosomes are dynamic actin-rich cell-matrix adhesion sites of migrating and invasive cells such as macrophages and osteoclasts, and are receiving increasing attention due to their possible involvement in physiological events such as monocyte extravasation and tissue transmigration, as well as pathological conditions such as atherosclerosis, osteoporosis and cancer metastasis. These structures were examined using three different fluorescence microscopy techniques which provide resolution below the diffraction limit: structured illumination microscopy, stimulated emission depletion microscopy and stochastic optical reconstruction microscopy have been used. In high resolution images, it is clearly visible that each podosome consists of an actin core surrounded by a protein-enriched ring, supporting the existing podosome model. However, these rings are polygonal structures rather than smooth circles. An analysis of the binding angles at corners reveals vinculin to have a dominant binding angle of around 115 degrees.

3334-Pos Board B439

Imaging Fluorescence Cross-Correlation Spectroscopy as a Tool to Study Cell-Membrane Organization

Jagadish Sankaran, Nirmalya Bag, Thorsten Wohland.

The structure of biological membranes has been investigated for many years. However, progress is hindered by the fact that putative domains are highly dynamic and their size is smaller than the optical diffraction limit and thus direct observations are difficult. Therefore, there is a need to develop new biophysical tools which can infer the existence of domains within membranes and can follow their development over time. We have introduced in the past a method called Imaging Total Internal Reflection-Fluorescence Correlation Spectroscopy (ITIR-FCS) using EMCCD or sCMOS cameras. ITIR-FCS allows the measurement of a large number (up to ~0.5 million) correlation curves at contiguous locations on cell membranes of live cells with millisecond time resolution. The spatial information within the data can be used to obtain information on the structure and organization of the membranes. This is achieved by calculating differences between the forward and backward cross-correlations between adjacent pixels A and B (CCF_{AB} - CCF_{BA}) or A, B, and C (CCF_{AB} - CCF_{CB}). The results can be depicted as histograms referred to as ΔCCF distributions. In this work we conduct measurements on supported lipid bilayers and cell membranes and perform simulations to demonstrate how ΔCCF distributions change characteristically with membrane complexity and structure. In particular, we demonstrate that domains with sizes below the diffraction limit have a characteristic broadening effect on the ΔCCF distributions. As an example we show that changes in membrane structure and organization of live neuroblastoma cells can be followed over the time course of an hour or more by way of ΔCCF distributions. To deal with large amount of data collected we developed an open source software, ImFCS, to calculate and fit the auto- and cross-correlation functions and depict the results in an imaging format.

3335-Pos Board B440

Probing Orientational Order of MHC Class I Protein and Lipids in Cell Membranes by Fluorescence Polarization-Resolved Microscopy Imaging

Alla Kress, Hubert Ranchon, Patrick Ferrand, Hervé Rigneault, Sophie Brasselet, Tomasz Trombik, Hai-Tao He, Didier Marguet.

Biomolecular orientational organization of lipids and proteins in plasma membrane is a crucial factor in biological processes where functions can be closely related to orientation and ordering mechanisms. The concept of transient nanosized phase separations in ordered and disordered domains, called ''lipid rafts'' is now widely accepted. Furthermore, the ordered domains are enriched in signalling proteins, which highlights the crucial impact of phase separation during the signalling processes. While this field has been so far largely addressed by studying the translational diffusion behaviour of membrane proteins by Single Molecule Tracking or Fluorescence Correlation Spectroscopy, only little is known about the orientational behaviour of signalling proteins in plasma membranes, mainly due to the lack of appropriate rigid fluorescent label which would be able to act as a proper orientation reporter. In this work we develop a fully polarization-resolved fluorescence imaging technique using a tuneable incident polarization state (''fluorescence polarimetry''), in combination with fluorescence anisotropy imaging, in order to provide orientational order information in very general cell membranes shapes.

We apply this technique to the measurement of quantitative orientational distribution of MHC Class I proteins in the plasma and nuclear membranes, benefiting from a rigidly attached GFP probe. The surrounding lipid orientational order in the plasma membrane is additionally probed using the fluorescent reporter di-8-ANEPPQ.The MHC Class I protein is found to be more ordered in the plasma membrane as compared to the nuclear membrane. Both MHC I and di-8-ANEPPQ orientational orders in the plasma membrane are furthermore seen to be highly affected by actin depolymerisation upon Latrunculin A treatment, with variations that indicate both a structural change in the membrane morphology and a disruption of MHC I - actin interactions.

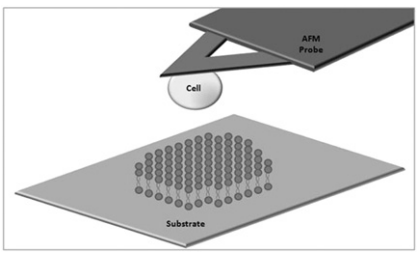
3336-Pos Board B441

A Combined Confocal-Total Internal Reflection Fluorescence (TIRF) Single-Cell Microscopy Investigation of CEACAM1 Dynamics

Laura N. Poloni, Christopher M. Yip.

The carcinoembryonic antigen-related cellular adhesion molecules (CEACAMs) represent a subset of the immunoglobulin superfamily of cell adhesion molecules that mediate intercellular adhesion. One member of this subset, CEACAM1, is particularly interesting since it is down-regulated in tumourigenic cell lines. CEACAM1 exists as an equilibrium between monomers and dimers; however, the spatial and temporal distribution upon cell-substrate contact is not known.

In order to determine CEACAM1 dynamics, a coupled confocal-total internal reflection fluorescence (TIRF)-atomic force microscopy (AFM) platform was used. Live YFP-CEACAM1 labelled cells were isolated on AFM tips for controlled positioning of cells. Confocal microscopy was used to map CEACAM1 receptors over the entire cell surface. Coupled TIRF microscopy monitored the free cell surface as it was brought into contact with a substrate (i.e. another cell, a model membrane, modified glass). Confocal and TIRF microscopy homoFRET measurements were used to determine the distribution of monomeric and dimeric CEACAM1 receptors prior to and upon cell-substrate contact. Through this understanding of how molecular organization affects intercellular binding and signal transduction, it may be possible to identify peptide or pharmacological drug strategies to create CEA CAM-focused therapies for cancer.



3337-Pos Board B442

Following Actin Fibers in 3D During Cell Migration in Collagen Matrices

Michelle A. Digman, Chi-Li Chiu, Jose S. Aguilar, Enrico Gratton.

Actin polymerization is a major mechanism for the production of the force necessary for cell migration in 2D. The polymerization of actin and its retrograde motion at the leading edge of cell moving in 2D has been studied in great detail as well as the interaction of actin with focal adhesions. When cells grow in 3D collagen matrices, the extending lamellipodial protrusion is more difficult to visualize and it is likely not relavent for the movement of the cell over large distances. We use the modulation tracking 3D method to accurately image the cell protrusion. This method is capable of producing detailed images of 3D structures at the nanoscale and at the same time measure diffusion and aggregation of molecules in these structures. In 3D, cells produce very long protrusions that presumably grab on the surrounding collagen fibers to propel the rest of the cell